Short Communication

A potential chiral derivatizing agent for 1,2-diglycerides

Philip E. Sonnet*[a], James E. Oliver[b], Roland M. Waters[c], Gregory King[a], Sini Panicker[b]

Abstract

Several chiral naphthylacetic acids have been prepared and evaluated as chiral derivatizing reagents for chiral alcohols. Fluorinated acids provide good general HPLC separations of chiral alcohols, especially 1,2-diglycerides. The separations and the ^{19}F-NMR shift differences of pairs of diastereomers are documented.

Keywords: Chiral alcohols; 1,2-Diglycerides; Fluoro-(1-naphthyl)acteic acids; Chiral derivatizing agents

1. Introduction

Hydrolysis of racemic or prochiral triglycerides of known structure by lipases (triacylglycerol hydrolases, EC 3.1.1.3) leads to 1,2-diglycerides whose configurations constitute useful baseline data for characterizing these enzymes. Conversely partial hydrolysis of natural triglycerides by lipases can be used to gain information about the configurations of the triglycerides. There has been a recent growth of interest in the structure of lipases [1], their applications in the fats and oils industries [2] and their remarkable ability to perform useful asymmetric syntheses and kinetic resolutions [3]. The interest specifically in understanding lipase mode of action is coupled to industrial interests in, for example, restructuring triglycerides and has focused attention on developing methods for determining 1,2-diglyceride configuration.

Recent reviews cite several complementary approaches that have been made in characterizing

1,2-diglycerides [4,5]. In our own research, direct derivatization of diglycerides followed by high-performance liquid chromatography (HPLC) appeared most efficient. Chiral derivatizing agents (CDA) that have been employed to date are isocyanates [6–8], the products from which are diastereomeric carbamates that are resolved by achiral silica gel columns. The experimental procedures reported involved several hours of heating followed by a column chromatography to purify the carbamates. Since one must use an excess of the chiral reagent to avoid possible fractionation of the diastereomers, much isocyanate remains as does the urea formed by reaction of the isocyanate with adventitious water.

Mosher's acid, α-methoxytrifluoromethylphenylacetic acid, or MTPA [9] has been a very valuable CDA. We prepared a triester from 1,2-diolein and MTPA, but it was not resolved by silica gel HPLC ($k' = 8.03$; 0.5% ethyl acetate/hexane as solvent). An analogous ester formed from (*S*)-*O*-acetylmandelic acid likewise was not resolved ($k' = 12.9$; 0.25% isopropanol/hexane). Esters formed from chiral acids often can be prepared at room temperature within a few hours using dicyclohexylcarbodiimide as a coupling agent in methylene chloride. The crude ester can be filtered from the urea byproduct and analyzed directly by HPLC. We report here the potential utility of fluoro-(1-naphthyl)acetic acid as a CDA for the resolution of 1,2-diglycerides and selected other chiral alcohols by HPLC and tabulate the observed ^{1}H- and ^{19}F-NMR shift differences.

2. Materials and methods

2.1. Reagents, materials and equipment

Melting points are uncorrected and were obtained on a Fisher-Johns hot stage. Silica gel plates of 0.25 mm thickness from Analtech, Inc. were used to monitor reactions by thin layer chromatography (TLC) with iodine visualization. Aldrich silica gel (230–400 mesh) was used for flash chromatography [10] and solvents were purchased from Burdick and Jackson or Aldrich Chemical Co. Tetrahydrofuran (THF) was dried by distillation from lithium aluminum hydride; other solvents were dried over 4A or 13A molecular sieves. Reagents were purchased from Aldrich Chemical Co. or Sigma Chemical Co. except *N*-fluorobenzenesulfonimide, which was purchased from Allied Signal (Buffalo, NY). The lipase of *Rhizopus oryzae* was a generous gift of Amano Co. (Troy, VA).

Gas-liquid chromatography (GLC) was performed with a Chrompack-Packard model 438A instrument using a flame ionization detector and a Supelcowax capillary column (0.25 mm × 30 m) and He carrier set to a 50:1 split ratio. High-performance liquid chromatography (HPLC) was accomplished with a Spectra-Physics SP8800 pump using a Supelcosil LC-Si column (4.6 mm × 25 cm) and a Spectra-Physics SP8480 UV detector at 280 nm. Infrared (IR) data were recorded with a Perkin-Elmer 1310 spectrophotometer using 1% solutions in CCl_4 or $CHCl_3$. The NMR spectra (^{1}H and ^{19}F) were determined as 0.01–0.02 M $CDCl_3$ solutions using a Bruker (GE) QE spectrometer operating at 300 and 282 MHz, respectively. Tetramethylsilane and fluorotrichloromethane were used as references. Mass spectra (MS) were obtained from a Finnegan model 4510 GC/MS; electron ionization spectra were collected at 70 eV and a source block temperature of 150°C. The molecular modeling work was conducted using the Sybyl (version 6.03, Tripos Associates, St. Louis, MO) software package on a Silicon Graphics Indigo 2 computer.

2.2. Synthesis of 1,2-diglycerides

The commercial triglyceride, trinonanoin or triolein, (2.0 g) was stirred magnetically with 50 ml of 0.05 M phosphate buffer at pH 7.00 and 0.50 g of *Rhizopus oryzae* lipase powder at room temperature for 2 h. The reaction mixture was extracted with 3 × 30 ml of hexane in order to recover diolein and the hexane extract was washed with 2 × 30 ml of H_2O. The organic phase was dried (Na_2SO_4) concentrated and purified by flash chromatography using 14% ethyl acetate/hexane. The dinonanoin was obtained by first shaking the reaction mixture with ether and then centrifugation to break the emulsion. This procedure was repeated to obtain an ether extract that was then processed as above for diolein. The diglycerides

were characterized by R_f value on TLC versus standards from Sigma Chemical Co.

2.3. Synthesis of substituted naphthylacetic acids

2.3.1. Synthesis of 2-(1'-naphthyl)propanoic acid **1a**

Naphthyl acetic acid (9.3 g, 0.050 mol) was added in portions through Gooch tubing to a stirred solution of lithium diisopropylamide (LDA) prepared from diisopropylamine (16.8 ml 0.120 mol) and *n*-butyllithium (44 ml of 2.5 M) in 75 ml of THF at −78°C. Hexamethylphosphoramide (HMPT) (10 ml) was added and the deep red solution was stirred for 1 h without external cooling. The mixture was cooled again and methyl iodide (7.5 ml 0.120 mol) was added. The resulting mixture was stirred at room temperature overnight and then worked up by dilution with 1 N HCI and extraction with ether. The extract was washed with water, dried ($MgSO_4$) and concentrated to a crystalline solid. Recrystallization from 220 ml of ethyl acetate/hexane (1:1 v/v) gave 6.25 g (78%) of **1a**: mp 146-147°C lit. 148–149°C [11]; IR 3680, 1725 cm^{-1}. Methyl ester: IR 3020, 1735 cm^{-1}; ^{1}H-NMR δ 7.27–8.13 (7H, Aryl H), 4.53 (1H, q, $J = 7.1$ Hz CH_3CH), 3.67 (3H, s, OCH_3) and 1.67 (3H d, $J = 7.1$ Hz, CH_3CH) ppm; ^{13}C NMR δ 175.85 (C=O), 137.33, 134.50, 131.80, 129.46, 128.22, 126.79 126.08, 124.98, 123.55 (Aryl C's), 52.56 (OCH_3) 41.81 (CH) and 18.69 (CH_3) ppm; GLC 240°C, 7.3 min; TLC (15% ethyl acetate/hexane) $R_f = 0.50$.

2.3.2. Synthesis of 2-fluoro-2-(1-naphthyl)-propanoic acid, **1c**, methyl ester

The methyl ester of **1a** (1.71 g, 8.0 mmol) was added as a solution in HMPT (2 ml) to a solution of lithioamide prepared from cyclohexylisopropylamide (1.5 ml, 9.2 mmol) and *n*-butyllithium (3.6 ml of 2.5 M) in THF (10 ml) at −78°C. The mixture was stirred cold for 0.5 h, then allowed to warm to room temperature for 1 h. The mixture was cooled again and *N*-fluorobenzenesulfonimide (2.80g, 8.9 mmol) was added in THF (3 ml). The resulting mixture was stirred for 1 h at ambient temperature and then worked up with ether. The organic extract was washed sequentially with 5% $NaHCO_3$, 2 N HCl and H_2O, dried ($MgSO_4$) and concentrated. Recrystallization from heptane gave 1.07 g (58%) of the methyl ester of **1c**: ^{1}H-NMR δ 7.49–8.25 (7.2H, Aryl H) 3.71 (3H, s, OCH_3) and 2.18 (3H, d, J_{HF} = 22.7 Hz, FC—CH_3) ppm; ^{13}C-NMR δ 172.58 173.08 (FC—*C*=O), 134.85, 134.65, 134.44, 131.04, 130.80 129.39, 127.29, 126.35, 125.17, 124.79, 124.63 (Aryl C's) 97.34, 93.70 (CF), 53.42 (OCH_3), 24.95 and 24.47 (FC—CH_3) ppm; GLC 240°C, 7.7 min. The free acid **1c**, was obtained by heating a solution of the ester (1.07 g, 4.6 mmol) in 5 ml of 50% aqueous methanol that was 1.8 M in KOH for 1 h. The mixture was worked up in the usual manner and the product recrystallized from isooctane to give 0.85 g (85%): mp 106–110°C (dec); IR 3680, 1730 cm^{-1}.

2.3.3. Synthesis of fluoro-(1-naphthyl)acetic acid, **1b**

This compound was synthesized by the procedure described in [12]: mp 146–149°C (dec). Methyl ester: mp 73–75°C (H_2O); IR 1750 cm^{-1}; ^{1}H-NMR δ 7.26–8.22 (Aryl H), 6.37 (2H, d, $J = 49.5$ Hz, HCF) and 3.76 (3H, s, OCH_3) ppm; ^{13}C-NMR δ 171.26 (C=O), 134.01, 132.10, 131.13, 129.49, 128.70, 126.76, 126.58 125.91, 125.21, 123.99 (Aryl C's), 57.42 and 52.26 (CF) ppm; ^{19}F-NMR δ −177.49 (d, $J = 49.5$ Hz, FCH) ppm; MS *m/e* 218 (26, M^+), 159 (100 $M-CO_2CH_3$) 133 [11]; GLC 240°C, 8.7 min.

2.3.4. Synthesis of fluoro-(2'-naphthyl)acetic acid **2**

The procedure followed was the general one previously reported [12]: mp 134–136°C (hexane/trace of ethyl acetate); MS *m/e* 218 (28, M^+), 159 (100 $M-CO_2CH_3$), 133 [9].

2.3.5. Preparation of diastereomeric esters

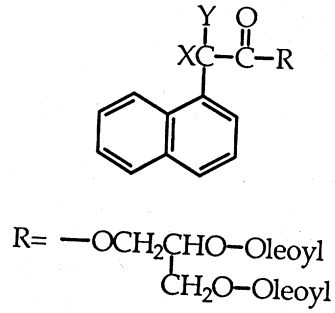
The alcohol or amine to be derivatized (20 μl) was added to 1 ml of CH_2Cl_2 containing **1b** (40 mg) dicyclohexylcarbodiimide (40 mg) and a trace of 4-dimethylaminopyridine. The mixture was allowed to stand overnight at ambient temperature. Hexane (1 ml) was added; the mixture was passed through a cotton plug, washed with 1.25 N NaOH and then passed through a plug of

Table 1
HPLC data for 1,2-diolein ester[a]

Compound	k[b]	α[c]
1a	2.81	1.089
1b	4.50	1.098
1c	3.06	1.075
2[d]	4.34	1.161

[a]5% Ethyl acetate/hexane.
[b]Capacity factor for diastereomer that eluted first.
[c]Ratio of ks.
[d]α-Fluoro-(2-naphthyl)acetic acid.

Na_2SO_4. The samples were now diluted for HPLC analysis. The HPLC analyses for 1,2-diolein esters are given in Table 1. Spectral and chromatographic data for various esters and amides of fluoro-(1-naphthyl)acetic acid are given in Table 2.



	X	Y
a	CH_3	H
b	H	F
c	CH_3	F

Scheme 1.

3. Results and discussion

Initially compounds **1a–c** were prepared and used to convert *rac*-1,2-diolein to a triester. The triesters are shown in Scheme 1. Syntheses of these acids are by conventional techniques and are detailed in the Materials and methods (Section 2.3).

The relevant HPLC data for the esters of **1a–c** with 1,2-diolein are given in Table 1. Encouraged

Table 2
HPLC and NMR shift data for fluoro-(1-naphthyl)acetate esters of chiral compounds

Compound	k[a]	α[b]	^{1}H-NMR (δ)[c]	^{19}F-NMR (δ)[c]
Alcohols				
2-Butanol[d]	2.81	1.028	0.020	0.173
2-Decanol[d]	2.17	1.110	0.034	0.252
2-Methyl-1-butanol[d]	2.53	1.043	0.005	0.254
1-Phenylethanol[d]	2.18	1.121	0.035	0.254
Citronellol[d]	2.56	1.00	0.00	0.00
Menthol[d]	1.42	1.00	0.068	0.302
1,2-Dinonanoin[e]	5.38	1.089	0.012	0.292
1,2-Dononanoin[e,f]	6.47	1.158	0.012	0.290
1,2-Didein[e]	4.50	1.098	0.012	0.292
Glycerol, 1-*n*-octyl-2-methyl diether[e]	5.36	1.090	0.006	0.009
Amine				
α-Phenylethylamine[g]	2.56	1.300	0.343	1.426

[a]Capacity factor for the diastereomer that eluted first.
[b]Ratio of capacity factors.
[c]ppm.
[d]1% Ethyl acetate/hexane.
[e]5% Ethyl acetate/hexane.
[f]CDA was fluoro-(2-naphthyl)acetic acid.
[g]10% Ethyl acetate/hexane.

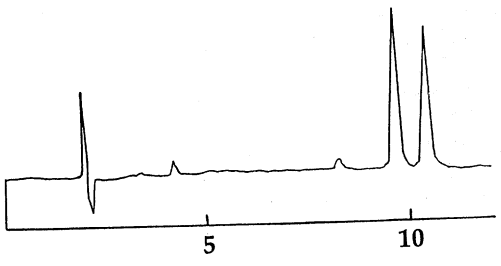


Fig. 1. Chromatogram (HPLC) of diastereomeric esters of fluoro-(1-naphthyl)acetic acid and 1,2-diolein (see Table 2).

by the separation of the dioleate diastereomers using the acid **1b** (Fig. 1), a number of other chiral alcohols were converted to esters with this acid. We also noted that esters of the 2-naphthyl analogue would likely give good separations of diastereomers as well. The data obtained for these compounds (Table 2) indicate that fluoro-(1-naphthyl)acetic acid might prove generally useful in analysis of a variety of chiral compounds and most importantly for 1,2-diglycerides.

Molecular modeling of the methyl esters of acids **1a–c** was performed with a desire to associate the magnitude of the ^{19}F shift differences with rotation conformer preferences. For each compound, rotation about the following three bonds was considered: the bond connecting the chiral carbon to the carbomethoxy group, the bond connecting the chiral carbon to the naphthyl group and the bond connecting the carbonyl carbon to the methoxy group. A systematic grid search was performed in which rotations of the three indicated bonds were generated in 15° increments. The conformers with the lowest energies were further refined by energy minimization to obtain the final structures. The Tripos molecular mechanics force field [13] and the MNDO semi-empirical quantum mechanical force field [14] produced nearly identical minimum energy conformations, with torsional angle variations of less than 5°

For all three compounds, the torsional angle associated with rotation about the carbonyl carbon-methoxy oxygen bond is 0° in the minimum energy conformations. For each compound, the potential energy minimum occurred with a conformation in which either the H (**1b**) or CH_3 (**1a**, **1c**) was slightly displaced from an eclipsed position with the carbonyl group. Relatively large differences in ^{19}F shifts might be expected, therefore, providing a substituent on the opposing asymmetric center is associated with strong anisotropy. Such a case exists with the amide formed from α-phenylethylamine. A biased diastereomer content of the diastereomer was obtained by chromatography, which showed that the first eluted diastereomer (major component of the mixture) had the CHF at lower field and the CHF at higher field. The principal rotamer, however suggests that the α-hydrogen ought to experience little shift difference and an assignment of elution orders vs. configurations must be deferred until the current effort to evaluate methods for obtaining configurationally pure derivatizing agents is completed. α-Fluorinated alkyl- and arylacetic acids, phenyl analogs of **1a–c**, have recently been prepared in high configurational purity [15,16]. Extension of these procedures to these compounds should be possible.

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